McLuckie, A., Tasker, S., Dhand, NK., Spencer, S., & Beatty, J. A. (2016). High prevalence of Felis catus gammaherpesvirus 1 infection in haemoplasma-infected cats supports co-transmission. *Veterinary Journal*, *214*, 117-121. https://doi.org/10.1016/j.tvjl.2016.06.001

16-00310

High prevalence of *Felis catus* gammaherpesvirus 1 infection in haemoplasma-infected cats supports co-transmission

A. McLuckie [a], S. Tasker [b], N.K. Dhand, [a] S. Spencer [b], J.A. Beatty [a,*]

[a] *Faculty of Veterinary Science and Marie Bashir Institute for Infectious Diseases and Biosecurity, School of Life and Environmental Sciences, The University of Sydney, NSW 2006, Australia*

[b] *The Feline Centre, Langford Veterinary Services and School of Veterinary Sciences, University of Bristol, Langford, Bristol BS40 5DU, UK*

[*]Corresponding author. Tel: +61 2 9351 3437.
E-mail address: julia.beatty@sydney.edu.au (J.A. Beatty).

Abstract

Felis catus gammaherpesvirus 1 (FcaGHV1), a potential feline pathogen, has been identified in domestic cats from USA, Asia-Pacific and Central Europe. Transmission of FcaGHV1 during territorial encounters, a route not typical for gammaherpesviruses, is suggested by risk factor analyses from some regions. The aim of this study was to investigate the relationship between FcaGHV1 detection and risk factors, including haemoplasma co-infections, among UK cats to better understand transmission and global distribution of FcaGHV1.

FcaGHV1 DNA was detected in blood samples from UK cats (11.56%; 95% confidence interval [CI] 7.47-16.84; *n* = 199). Logistic regression analyses showed that entire male cats were more likely to be FcaGHV1 positive than neutered male cats (odds ratio 3.60; 95% CI 1.22-10.46). Samples positive for DNA from any of three haemoplasma species had 19 times greater odds for testing positive for FcaGHV1 than haemoplasma negative cats in multivariable analyses after adjusting for age, sex and neuter status. Domestic cats in the UK can be infected with FcaGHV1, confirming that this virus is globally endemic. The identification of neuter status as a risk factor for FcaGHV1 detection provides further evidence to support transmission of this virus during territorial encounters and co-transmission with haemoplasmas is suggested.

Keywords: Co-pathogen; Feline; Gammaherpesvirus; Haemoplasma; Transmission

Introduction

Felis catus gammaherpesvirus 1 (FcaGHV1), the first gammaherpesvirus (GHV) to be identified in domestic cats, has been detected in blood samples from cats in the USA, Australia, Singapore and, most recently, Central Europe (Beatty et al., 2014; Troyer et al., 2014; Ertl et al., 2015). The consequences of FcaGHV1 for feline health are yet to be elucidated. Herpesviruses establish latent infections in their hosts that persist lifelong. In other species, gammaherpesvirus infections are typically clinically silent unless there is a failure of immunological containment resulting from infection of a non-adapted host or immune dysfunction in the natural host (Ackermann, 2006). In such circumstances, GHVs cause a range of lymphoproliferative and neoplastic diseases that are frequently fatal. For example, in the setting of HIV-AIDS, a causal role for Epstein-Barr virus (EBV) in most HIV-associated lymphomas is well established with some subtypes, such as diffuse large B-cell lymphoma, almost invariably harbouring latent EBV (Raphael et al., 2008; Cesarman, 2011). In cats, the recognition that infection with the lentivirus, feline immunodeficiency virus (FIV) confers an increased risk of the development of similar high-grade B cell lymphomas prompted the search for a feline GHV (Beatty et al., 2012). The pathogenic potential of FcaGHV1 has veterinary and one health implications; susceptibility of domestic cats to natural infection with both FcaGHV1 and FIV suggests a novel, spontaneous model of GHV-induced disease.

To date, information on virus-host interactions has come from epidemiological investigations that have identified sex (male), age (adult) and health status (sick) as risk factors for FcaGHV1 detection (Beatty et al., 2014; Troyer et al., 2014; Ertl et al., 2015). Significant associations between FcaGHV1 and coinfection with FIV, feline leukaemia virus or

haemoplasmas '*Candidatus* Mycoplasma haemominutum (CMhm)' and *Mycoplasma haemofelis* (Mhf) are found in some, but not all, regions. We investigated, for the first time in cats from UK, associations between FcaGHV1 detection and age, sex, neuter status, breed and coinfection infection with three haemoplasma species: CMhm, Mhf and '*Candidatus* Mycoplasma turicensis (CMt)'.

Materials and methods

Samples for molecular epidemiological studies

This study was approved by the University of Bristol's Animal Welfare and Ethical Review Board (No. 14/017, 22 May 2014). Residual DNA from whole blood submitted to The Molecular Diagnostic Unit, Langford Veterinary Services, University of Bristol, for PCR testing for haemoplasmas was available for study. All samples originated from cats domiciled in the UK. The clinical indication for haemoplasma testing was determined by the veterinarian submitting the sample. Age, sex, neuter status and breed data were available for all samples. PCR testing for CMhm, Mhf and CMt was carried out, as described previously (Table 1; Peters et al., 2008). DNA from 90 samples testing positive for at least one haemoplasma species and 109 haemoplasma negative samples was shipped to Australia on ice. A qPCR targeting the glycoprotein B gene of FcaGHV1 was performed on the DNA samples with the operator (AM) blinded to sample data (age, sex, neuter status, breed, results of haemoplasma PCR; Troyer et al., 2014). For FcaGHV1 qPCR, amplification efficiencies were 95 – 105% and r^2 >0.99. Triplicate reactions containing >3 copies/reaction were considered positive for FcaGHV1.

Prevalence and risk factors

All statistical analyses were conducted using the SAS Statistical Program (SAS version 9.4 2002-2012, SAS). FcaGHV1 prevalence and 95% exact confidence intervals were calculated using the FREQ procedure in SAS with a binomial option. To identify risk factors for FcaGHV1, descriptive analyses were conducted which included the creation of frequency tables for categorical variables and calculation of summary statistics for age. Contingency tables of categorical variables with FcaGHV1 were created to make a preliminary evaluation of association of categorical risk factors with the outcome variable (Table 2). The association between sex and FcaGHV1 was evaluated, both overall (ignoring neuter status) and after stratifying by neuter status. Univariable logistic regression analyses were then conducted using SAS Logistic procedure with the assistance of UniLogistic SAS macro to evaluate unconditional association of all explanatory variables (both categorical and quantitative) with FcaGHV1 (Dhand, 2010). The assumption of linearity of age, the only quantitative variable, was evaluated by creating a spline facilitated by the PSPLINET macro[1]. As this assumption was invalid, we categorised age into three groups for further analyses; ≤2 years, >2-≤10 years and >10 years. Multivariable logistic regression analyses were finally conducted using the MultiLogistic SAS macro[2] to evaluate the associations between explanatory variables after adjusting for each other and potential confounders (age and sex). First order interactions between variables in the final models were tested and retained if significant. Model fit was evaluated using the Hosmer-Lemeshow goodness-of-fit test. Two separate multivariable models were built for CMhm and hemoplasma (any) detection because these variables were highly correlated.

[1] See: http://biostat.mc.vanderbilt.edu/twiki/bin/view/Main/SasMacros (accessed 3 June 2016).
[2] See: http://sydney.edu.au/vetscience/biostat/macros/index.shtml (accessed 3 June 2016).

Results

Samples from 199 cats were available for FcaGHV1 testing (Table 2). The median age of this population was 6 years (range, 0.3 to 17 years; interquartile range, 7 years). Among 90 haemoplasma infected cats, single infections with CMhm, Mhf or CMt were identified in 71 (78.9%), nine (10%) and four (4.4%) cats and dual infections were detected in six cats (6.7%; CMhm/Mhf, *n* = 3; CMhm/CMt, *n* = 1; Mhf/CMt, *n* = 2). Overall, the prevalence of FcaGHV1 DNA detection was 11.56% (95% CI, 7.47-16.84).

In crude analyses, males were more likely to be FcaGHV1 positive than females (odds ratio, 4.45; 95% CI, 1.22-28.73) and entire cats had greater odds of being FcaGHV1 positive than neutered cats (Table 3). In stratified analysis, entire male cats had 3.57 times increased odds of FcaGHV1 detection than neutered male cats (Fisher's exact test, $P = 0.027$), but such an odds ratio could not be calculated for female cats, as none of the neutered cats and only two of the entire cats tested positive for FcaGHV1. However, the Fisher's exact test conducted to evaluate the association of neutered and entire female cats with FcaGHV1 was marginally non-significant ($P = 0.07$).

Other univariable analyses identified significant associations between FcaGHV1 detection and concurrent haemoplasma (any) or CMhm DNA detection (Table 3). A total of 21/23 of FcaGHV1 positive cats (91%) were coinfected with at least one haemoplasma spp., while 69/176 of FcaGHV1 negative cats (39%) harboured haemoplasma DNA. No cat < 2 years of age was infected with FcaGHV1. The risk of FcaGHV1 detection increased with age and

plateaued at approximately 5 years, although this association was not statistically significant (Fig. 1).

In multivariable analyses, separate models were built because hemoplasma (any) and CMhm detection were highly correlated (Table 4). In these models, the odds ratio for FcaGHV1 detection in cats testing positive for haemoplasma (any) was 19.14. Similarly, in cats testing positive for the individual species CMhm or Mhf, the risk of FcaGHV1 detection was statistically significant, with odds ratios of 14.96 and 9.24, respectively. Model fits of both multivariable models were adequate (Hosmer-Lemeshow goodness-of-fit $P = 0.68$ and $P = 0.57$, respectively; $P > 0.05$ suggests an adequate model fit).

Discussion

FcaGHV1 infection was commonly detected among pet cats from the UK, confirming that this virus is widely endemic. The prevalence of FcaGHV1 detection reported here (11.6%) is comparable with results from studies of domestic cats in the USA (16%), Australia (11.5%), Singapore (9.6%) and central Europe (16.2%; Beatty et al., 2014; Troyer et al., 2014; Ertl et al., 2015).

Our observation that FcaGHV1-infected cats were at least 2 years of age is consistent with previous studies identifying age as a risk factor for FcaGHV1 infection and together these data support horizontal rather than vertical transmission of this virus. Almost all FcaGHV1 infected cats were concurrently infected with one or more haemoplasma species, an association that remained significant even after adjusting for age, sex and neuter status. This relationship

153 also held true for two individual haemoplasma species, CMhm and Mhf, but not for CMt. The
154 relative prevalence of haemoplasma infections should be considered when interpreting these
155 results. The infrequent identification in our study of CMt infection, in line with a large UK study
156 that determined the prevalence of CMhm, Mhf and CMt to be 11.2%, 2.8% and 1.7%,
157 respectively (Peters et al., 2008), may have precluded the detection of any association between
158 this species and FcaGHV1 coinfection. Inclusion of a greater number of CMt infected cats in
159 future studies will assist in better defining the relationship between this haemoplasma species
160 and FcaGHV1 coinfection. The predominance of CMhm infections here, expected from
161 established haemoplasma epidemiological data (Barker and Tasker, 2013), underlies the
162 association between the detection of CMhm and any haemoplasma DNA, and the consequent
163 requirement for two multivariable models to be built in this study.
164
165 Epidemiological relationships between FcaGHV1 and coinfections or sex, identified
166 using traditional statistical approaches as well as structural equation models, point to a potential
167 role for aggressive male behaviours in FcaGHV1 transmission (Beatty et al., 2014; Carver et al.,
168 2015; Ertl et al., 2015). The association between hemoplasma infection and male sex and/or
169 retrovirus status, particularly FIV, reported in some studies supports a role for inter-cat
170 aggression in haemoplasma transmission (Luria et al., 2004; Sykes et al., 2008; Georges et al.,
171 2012; Ghazisaeedi et al., 2014). One Swiss study demonstrated that subcutaneous inoculation of
172 CMt-containing blood resulted in transmission of infection, whereas the same inoculation
173 method using CMt-containing saliva did not (Museux et al., 2009). This suggests that
174 hemoplasma transmission by social contact (saliva via mutual grooming etc.) is less likely than
175 transmission by aggressive interaction (blood transmission during a cat bite incident). However,

176 a recent study found evidence of horizontal transmission of CMhm, but not Mhf, by direct
177 contact between cats in the absence of aggressive interaction and arthropod vectors (Lappin,
178 2014).
179
180 Our observation that entire male cats are at increased risk of FcaGHV1 infection
181 compared with neutered males provides a novel line of evidence to support FcaGHV1
182 transmission during aggressive encounters. Inter-cat aggression resulting in injury is more
183 common between males than females and this behaviour is reduced or abolished by castration
184 (Hart and Barrett, 1973; Dards, 1983; Lindell et al., 1997). Interestingly, in contrast to the
185 current study, neuter status was not a risk factor for FcaGHV1 infection in a large population of
186 cats from Germany and Austria that used a similar proportion of entire cats to that studied here
187 (Ertl et al., 2015). This difference mirrors that identified previously in Singapore, where sex was
188 not a risk factor for FcaGHV1 or for FIV infection, a virus for which the major mode of
189 transmission is typically fighting. The predominant mode of FcaGHV1 transmission may thus be
190 impacted by regional differences.
191
192 Other explanations may account for the frequent observation of FcaGHV1 and
193 haemoplasma coninfections. FIV, a risk factor for both FcaGHV1 and for haemoplasmas
194 detection, can cause immune dysfunction, which may facilitate increased replication of both
195 agents above the limit of detection of diagnostic assays (Macieira et al., 2008). Data on the FIV
196 status of this population were not available. The use of a convenience sample of clinical
197 specimens submitted for haemoplasma PCR testing is a potential limitation of our study.
198 However, since clinicians submitting the samples had no knowledge of the FcaGHV1 status of

cats, we consider selection bias to be unlikely. Indications for haemoplasma testing include anaemia, which although not immediately linked to known gammaherpesvirus pathologies, could be indirectly associated with gammaherpesvirus-associated neoplasia, thereby skewing the results.

Conclusions

Domestic cats in the UK can be infected with FcaGHV1, confirming that investigation of the pathogenic potential of this novel feline virus is relevant for feline health and welfare globally. The identification of neuter status as a risk factor for FcaGHV1 detection provides further evidence to support transmission of this gammaherpesvirus during aggressive territorial encounters; co-transmission with haemoplasmas is suggested.

Conflict of interest statement

None of the authors of this paper has a financial or personal relationship with other people or organisations that could inappropriately influence or bias the content of the paper.

Acknowledgements

This study was supported by the Dugdale Guy Peele bequest to the University of Sydney. We thank our colleagues Chris Helps, David Morris and Chelsea Hicks of Langford Veterinary Services and School of Veterinary Sciences, University of Bristol for their assistance in sourcing samples for use in this study.

References

Ackermann, M., 2006. Pathogenesis of gammaherpesvirus infections. Veterinary Microbiology 113, 211-222.

Barker, E., Tasker, S., 2013. Haemoplasmas: Lessons learnt from cats. New Zealand Veterinary Journal 61, 184-192.

Beatty, J.A., Troyer, R.M., Brewster, C., Rovnak, R., Barrs, V.R., Quackenbush, S.L., VandeWoude, S., 2012. Feline immunodeficiency virus (FIV)-associated lymphoma. Is a gammaherpesvirus involved? (Abstract). In: Proceeding of the 2nd International Society for Companion Animal Infectious Diseases symposium. San Francisco, CA, USA.

Beatty, J.A., Troyer, R.M., Carver, S., Barrs, V.R., Espinasse, F., Conradi, O., Stutzman-Rodriguez, K., Chan, C.C., Tasker, S., Lappin, M.R., et al., 2014. Felis catus gammaherpesvirus 1; a widely endemic potential pathogen of domestic cats. Virology 460, 100-107.

Carver, S., Beatty, J.A., Troyer, R.M., Harris, R.L., Stutzman-Rodriguez, K., Barrs, V.R., Chan, C.C., Tasker, S., Lappin, M.R., VandeWoude, S., 2015. Closing the gap on causal processes of infection risk from cross-sectional data: structural equation models to understand infection and co-infection. Parasites and Vectors 8, 1-7.

Cesarman, E., 2011. Gammaherpesvirus and lymphoproliferative disorders in immunocompromised patients. Cancer Letters 305, 163-174.

Dards, J.L., 1983. The behaviour of dockyard cats – intercations of adult males. Applied Animal Ethology 10, 133-153.

Dhand, N.K., 2010. UniLogistic: a SAS macro for descriptive and univariable logistic regression analyses. Journal of Statistical Software 35, 1-15.

Ertl, R., Korb, M., Langbein-Detsch, I., Klein, D., 2015. Prevalence and risk factors of gammaherpesvirus infection in domestic cats in Central Europe. Virology Journal 12, 146.

Georges, K., Ezeokoli, C., Auguste, T., Seepersad, N., Pottinger, A., Sparagano, O., Tasker, S., 2012. A comparison of real-time PCR and reverse line blot hybridization in detecting feline haemoplasmas of domestic cats and an analysis of risk factors associated with haemoplasma infections. BMC Veterinary Research 8, 103.

Ghazisaeedi, F., Atyabi, N., Zahrai Salehi, T., Gentilini, F., Ashrafi Tamai, I., Akbarein, H., Tasker, S., 2014. A molecular study of hemotropic mycoplasmas (hemoplasmas) in cats in Iran. Veterinary Clinical Pathology 43, 381-386.

Hart, B.L., Barrett, R.E., 1973. Effect of castration on fighting roaming and urine spraying in adult male cats. Journal of the American Veterinary Medical Association 163, 290-292.

Lappin, M.R., 2014. Feline haemoplasmas are not transmitted by Ctenocephalides felis, In: Proceedings of the 9th Symposium of the Companion Vector-Borne Diseases World Forum, Lisbon, Portugal.

Lindell, E.M., Erb, H.N., Houpt, K.A., 1997. Intercat aggression: A retrospective study examining types of aggression, sexes of fighting pairs, and effectiveness of treatment. Applied Animal Behaviour Science 55, 153-162.

Luria, B.J., Levy, J.K., Lappin, M.R., Breitschwerdt, E.B., Legendre, A.M., Hernandez, J.A., Gorman, S.P., Lee, I.T., 2004. Prevalence of infectious diseases in feral cats in Northern Florida. Journal of Feline Medicine and Surgery 6, 287-296.

Macieira, D.B., de Menezes, R., Damico, C.B., Almosny, N.R., McLane, H.L., Daggy, J.K., Messick, J.B., 2008. Prevalence and risk factors for hemoplasmas in domestic cats naturally infected with feline immunodeficiency virus and/or feline leukemia virus in Rio de Janeiro - Brazil. Journal of Feline Medicine and Surgery 10, 120-129.

Museux, K., Boretti, F.S., Willi, B., Riond, B., Hoelzle, K., Hoelzle, L.E., Wittenbrink, M.M., Tasker, S., Wengi, N., Reusch, C.E., et al., 2009. In vivo transmission studies of 'Candidatus Mycoplasma turicensis' in the domestic cat. Veterinary Research 40, 45.

Peters, I.R., Helps, C.R., Willi, B., Hofmann-Lehmann, R., Tasker, S., 2008. The prevalence of three species of feline haemoplasmas in samples submitted to a diagnostics service as determined by three novel real-time duplex PCR assays. Veterinary Microbiology 126, 142-150.

Raphael, M., Said, J., Borisch, B., Cesarman, E., Harris, N.L., 2008. Lymphomas associated with HIV infection, In: WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues, Fourth Ed. IARC Press, Lyon, France, pp340-342.

Sykes, J.E., Terry, J.C., Lindsay, L.L., Owens, S.D., 2008. Prevalences of various hemoplasma species among cats in the United States with possible hernoplasmosis. Journal of the American Veterinary Medical Association 232, 372-379.

Troyer, R.M., Beatty, J.A., Stutzman-Rodriguez, K.R., Carver, S., Lozano, C.C., Lee, J.S., Lappin, M.R., Riley, S.P.D., Serieys, L.E.K., Logan, K.A., et al., 2014. Novel Gammaherpesviruses in North American Domestic Cats, Bobcats, and Pumas: Identification, Prevalence, and Risk Factors. Journal of Virology 88, 3914-3924.

Table 1

Details of the quantitative PCR assays used for the detection of the three haemoplasma species and feline 28S rDNA (Peters et al., 2008), and *Felis catus* gammaherpesvirus 1 (Troyer et al., 2014).

Target	Forward primer	Reverse primer	5' fluorophore	Probe	3' quencher	Product size (bp)
Mhf	GTGCTACAATGG CGAACACA	TCCTATCCGAACT GAGACGAA	FAM	TGTGTTGCAAACCAG CGATGGT	BHQ-1	80
CMhm	TGATCTATTGTKA AAGGCACTTGCT	TTAGCCTCYGGTG TTCCTCAA	FAM	TTCAATGTGTAGCGG TGGAATGCGT	BHQ-1	135
CMt	AGAGGCGAAGGC GAAAACT	CTACAACGCCGA AACACAAA	FAM	CGTAAACGATGGGT ATTAGATGTCGGGAT	BHQ-1	138
Feline 28S rDNA	AGCAGGAGGTGT TGGAAGAG	AGGGAGAGCCTA AATCAAAGG	Texas Red	TGGCTTGTGGCAGCC AAGTGT	BHQ-2	100
FcaGHV1	ACATCTTCACTGG ACAACTGG	GTGCATTTGATGT CCTGACTG	FAM	TGAACAGCTGAGTCT CTACAAGTCTCCA	TAMRA	113

Mhf, *Mycoplasma haemofelis*; CMhm, *Candidatus* Mycoplasma haemominutum; CMt, *Candidatus* Mycoplasma turicensis; FcaGHV1, *Felis catus* gammaherpesvirus 1.

Table 2

Contingency tables of categorical variables with outcome, FcaGHV1 detection.

		FcaGHV1 status			
Variables	Categories	Positive	Negative	Total	% Positive
Haemoplasma (any)	Negative	2	107	109	2
	Positive	21	69	90	23
CMhm	Negative	4	120	124	3
	Positive	19	56	75	25
Mhf	Negative	20	165	185	11
	Positive	3	11	14	21
CMt	Negative	21	171	192	11
CMt	Positive	2	5	7	29
Age (years)	≤2	2	34	36	6
	>2-≤10	11	83	94	12
	>10	6	33	39	15
Sex	Male	18	99	117	15
	Female	2	49	51	4
Neuter status	Entire	10	30	40	25
	Neutered	10	118	128	8
Breed	Crossbred	16	130	146	11
	Purebred	2	22	25	8

FcaGHV1, *Felis catus* gammaherpesvirus 1; CMhm, *Candidatus* Mycoplasma haemominutum; Mhf, *Mycoplasma haemofelis;* CMt, *Candidatus* Mycoplasma turicensis.

Table 3

Univariable logistic regression analyses to evaluate unconditional association between explanatory variables and outcome, *Felis catus* gammaherpesvirus 1 (FcaGHV1) detection.

Parameters	Categories	b [a]	SE [b]	Odds-ratio	95% CI	*P* [c]
Intercept	Positive vs. negative	-3.98	0.71			
Haemoplasma (any)		2.79	0.76	16.28	4.58-103.80	<0.0001
Intercept	Positive vs. negative	-3.40	0.51			
CMhm		2.32	0.57	10.18	3.63-36.36	<0.0001
Intercept	Positive vs. negative	-2.11	0.24			
Mhf		0.81	0.69	2.25	0.48-7.96	0.273
Intercept	Positive vs. negative	-2.10	0.23			
CMt		1.18	0.87	3.26	0.45-16.19	0.212
Intercept	2 to ≤10 vs. ≤2	-2.83	0.73			
Age	>10 vs. ≤2	0.81	0.80	2.25	0.57-15.06	0.362
		1.13	0.85	3.09	0.66-22.13	
Intercept	Male vs. female	-3.20	0.72			
Sex		1.49	0.77	4.45	1.22-28.73	0.021
Intercept	Entire vs. neutered	-2.47	0.33			
Neuter status		1.37	0.49	3.93	1.49-10.45	0.006
Intercept	Purebred vs. crossbred	-2.09	0.27			
Breed		-0.30	0.78	0.74	0.11-2.85	0.69

CMhm, *Candidatus* Mycoplasma haemominutum*;* Mhf, *Mycoplasma haemofelis;* CMt, *Candidatus* Mycoplasma turicensis; CI, confidence interval; SE Standard error

[a] Parameter estimate.

[b] Standard error of the parameter estimate.

[c] Based on likelihood ratio chi-square test.

Table 4

Final multivariable logistic regression models to identify risk factors for *Felis catus* gammaherpesvirus 1 (FcaGHV1) detection. Model 1 includes haemoplasma (any) while Model 2 includes and CMhm and Mhf.

Variables	Categories	b [a]	SE [b]	Odds ratio	LCL-UCL	*P*
Model 1 incorporating haemoplasma (any)						
Intercept		-5.44	1.38			
Haemoplasma (any)	Positive vs. negative	2.95	1.07	19.14	3.52-357.99	0.000
Neuter status	Entire vs. neutered	1.12	0.58	3.06	0.99-9.69	0.052
Age	2 to ≤10 vs. ≤2	0.19	0.91	1.20	0.22-9.44	0.941
	>10 vs. ≤2	0.32	0.97	1.38	0.22-11.77	
Sex	Male vs. female	0.84	0.85	2.32	0.51-16.74	0.295
Model 2 incorporating CMhm and Mhf						
Intercept		-5.02	1.22			
CMhm	Positive vs. negative	2.71	0.86	14.96	3.40-112.00	0.000
Mhf	Positive vs. negative	2.22	0.97	9.24	1.40-76.35	0.022
Neuter status	Entire vs. neutered	1.18	0.58	3.25	1.04-10.48	0.042
Age	2 to ≤10 vs. ≤2	0.15	0.91	1.17	0.22-9.07	0.972
	>10 vs. ≤2	0.23	0.98	1.26	0.20-10.77	
Sex	Male vs. female	0.66	0.88	1.94	0.39-14.49	0.437

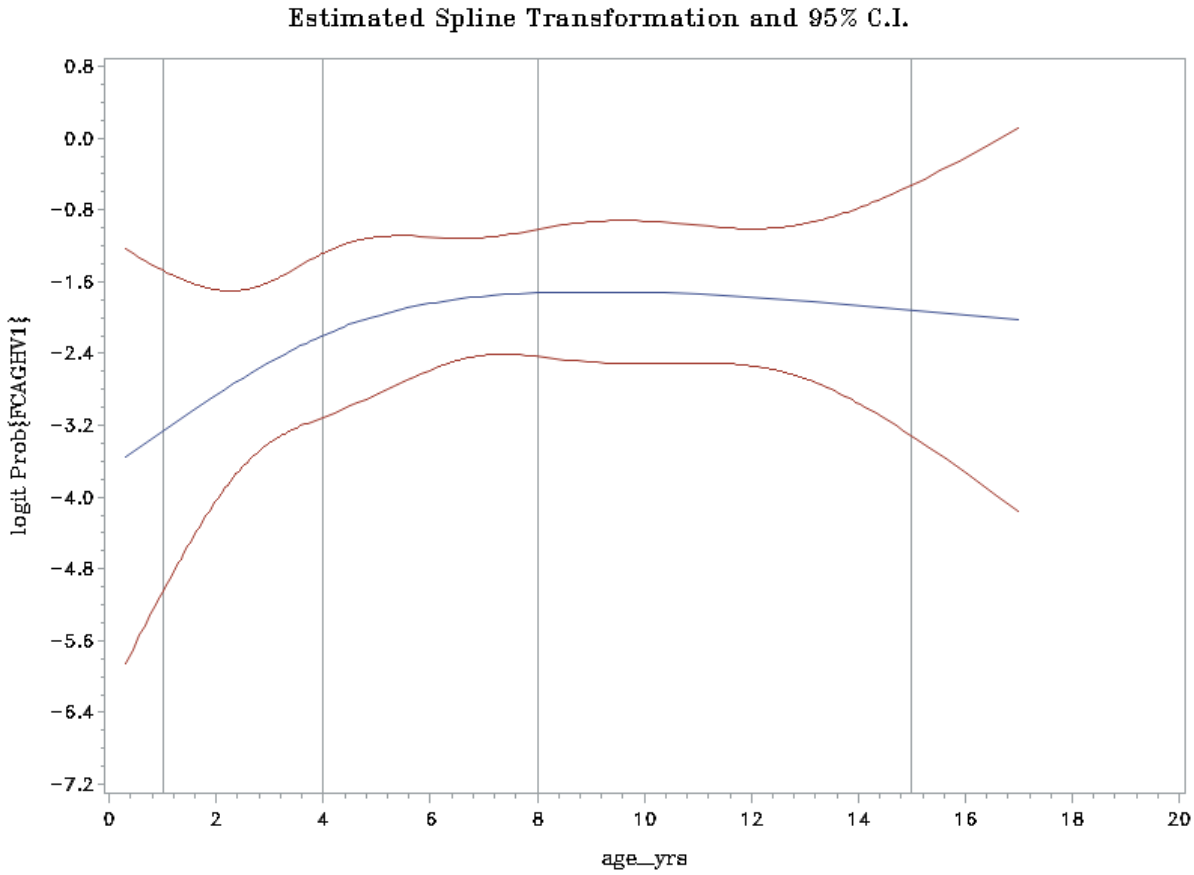
CMhm, *Candidatus* Mycoplasma haemominutum; Mhf, *Mycoplasma haemofelis*; LCL, lower control limit; UCL, upper control limit.

[a] Parameter estimate.

[b] Standard error of the parameter estimate.

Figure legend

Fig. 1. Estimated spline transformation and 95% confidence limits of the association between age included a quantitative variable and *Felis catus* gammaherpesvirus 1 (FcaGHV1) detection among pet cats in the UK. Reference lines depict knots in the spline.


Estimated Spline Transformation and 95% C.I.
logit Prob{FCAGHV1}
0.8
0.0
-0.8
-1.6
-2.4
-3.2
-4.0
-4.8
-5.6
-6.4
-7.2
0
2
4
6
8
10
12
14
16
18
20
age_yrs